duces different outputs depending on when and where in the dendritic tree the input occurred. This in turn could allow the cell to recognize specific input patterns[15]. When such patterns (giving rise to subthreshold inputs at the apical dendrites) are associated with a strong input (sufficient to trigger a backpropagating action potential), the cell would signal this by firing a burst. One can speculate that such transformations—in which an irregular temporal pattern of presynaptic spikes is transformed into a pattern of bursts by the postsynaptic neuron—might form the cellular basis for the emergence of large functional assemblies of neurons. The dominance of synchronized bursting of neurons in different layers and different columns produced during intense association of ascending and descending information may underlie episodic perceptual binding at the cortical level. Burst behavior, in turn, may extend the temporal window during which a given cortical cell can detect the arrival of a combination of inputs in distinct dendritic compartments and promote the boosting and eventually the strengthening of otherwise subliminal synaptic influences.

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BMPs: time to murder and create?

Gordon Fishell

The olfactory epithelium produces new neurons throughout life. Shou ***et al.*** show that BMPs can inhibit this process by inducing degradation of the transcription factor MASH1.

When it comes to proliferation in the mature organism, neurons on the whole are a timid bunch. During embryogenesis, massive proliferation occurs in both the central and peripheral nervous system, but this ends by or soon after birth, and there is very little neurogenesis in the adult. One exception, however, is the olfactory epithelium, where new olfactory receptor neurons (ORNs) continue to be formed throughout life[1,2]. Work from many laboratories has suggested that the generation of new ORNs is a dynamically regulated process. For instance, their rate of production is dramatically increased in response to injury[3,4] suggesting that neurogenesis in the intact epithelium may normally be repressed. The signal mediating this repression has so far remained elusive, but a paper on page 339 of this issue suggests that the culprit may be a bone morphogenic protein (BMP).

BMPs are a large family of secreted growth factors, the original members of which were identified by their ability to promote bone growth. Our view of their myriad functions continues to expand, and BMPs have now been shown to act on most tissues of the body. In the developing nervous system, for instance, BMPs inhibit neural induction, dorsalize the spinal cord and promote cell death in the hindbrain[5].

Their presence in the olfactory epithelium and their inhibitory effects in other parts of the nervous system suggested to Shou and colleagues that BMPs might also be promising candidates for mediating the inhibition of ORN development. To test this possibility, the authors used a neuronal colony-forming assay, in which the various steps of ORN proliferation and differentiation are recapitulated in culture. If left unperturbed for six days, olfactory epithelial cultures give rise to mixed colonies containing both neuronal progenitors and differentiated ORNs; the cells from which the colonies arise are thought to be the stem cells that give rise to new neurons *in vivo*[6]. The authors found, remarkably, that the addition of BMP2, 4 or 7 to these cultures completely blocks the appearance of colonies.

The progression from stem cell to differentiated neuron is a multi-step process with at least two defined intermediate stages[2]. To determine where in this process BMPs might act, the authors added BMPs at different times and found that the block to ORN production occurred within the first twenty-four hours. This is at least three days before differentiated ORNs begin to appear, suggesting that BMPs must block a relatively early stage in the ORN lineage. Consistent with this, early exposure to BMPs greatly reduced the level of proliferation (as measured by incorporation of [^{3}H]thymidine by neuronal progenitor cells), suggesting that BMPs act on a still-proliferating precursor rather than on postmitotic neurons. BMPs do not cause an immediate increase in cell death, although apoptotic cell death does occur later.

How might BMPs inhibit development of ORN precursors? The authors investigated the possibility that it might act through the transcription factor MASH1 (mammalian achaete-scute homolog 1), which is expressed at an early stage in the olfactory receptor lineage[7]. MASH1 was an attractive candidate because mutant mice lacking this protein show a phenotype that is very reminiscent of the BMP-treated cultures; mature ORNs are almost totally absent from the olfactory epithelium, which instead shows a high level of apoptotic cell death[8]. The authors therefore examined the effect of BMP treatment on MASH1 expression in their cultures. Within sixty minutes of exposure to BMPs, the number of MASH1-expressing cells fell by fifty percent, with a maximal decrease seen after

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two hours. This rapid loss of MASH1 cannot be explained simply by cessation of new synthesis, because the half-life of the protein in these cells (as measured using cycloheximide to block protein synthesis) is at least four hours. Instead, MASH1 must be actively degraded; consistent with this, its disappearance can be prevented by pharmacological inhibitors of proteasome activity, suggesting that BMP causes MASH1 protein to be somehow targeted for proteasome-mediated proteolysis. One trivial explanation for the results might be that MASH1 degradation reflects the nonspecific proteolysis that precedes apoptotic cell death. This seems unlikely, however, because cell death does not occur until much later (20 hours). A more direct test would be to show that the disappearance of MASH1 is independent of caspases (which mediate proteolysis during apoptosis), but this experiment may be difficult, given the diversity of caspases and the complexity of their regulation.

The findings of Shou and colleagues suggest the following scenario (Fig. 1a): BMPs induce a rapid proteasome-mediated degradation of MASH1 protein at an early stage in the ORN lineage. The progenitors that lose MASH1 cease proliferation and ultimately undergo apoptosis, terminating the lineage before differentiated ORNs can be generated. To prove this model, one would ideally like to block MASH1 proteolysis and show that proliferation and differentiation can then proceed even in the presence of BMPs. However, this experiment is not straightforward; although proteasome inhibitors can be used for short-term studies on MASH1 degradation, prolonged exposure is toxic to these cultures, precluding any study of the longer-term consequences for proliferation or differentiation. It may eventually be possible to identify and mutate the sites in MASH1 that cause it to be targeted for proteolysis, but this would involve substantial further work. Hence, although the phenotype of the MASH1-deficient mice is certainly suggestive of a causal relationship, it may be some time before this can be tested directly.

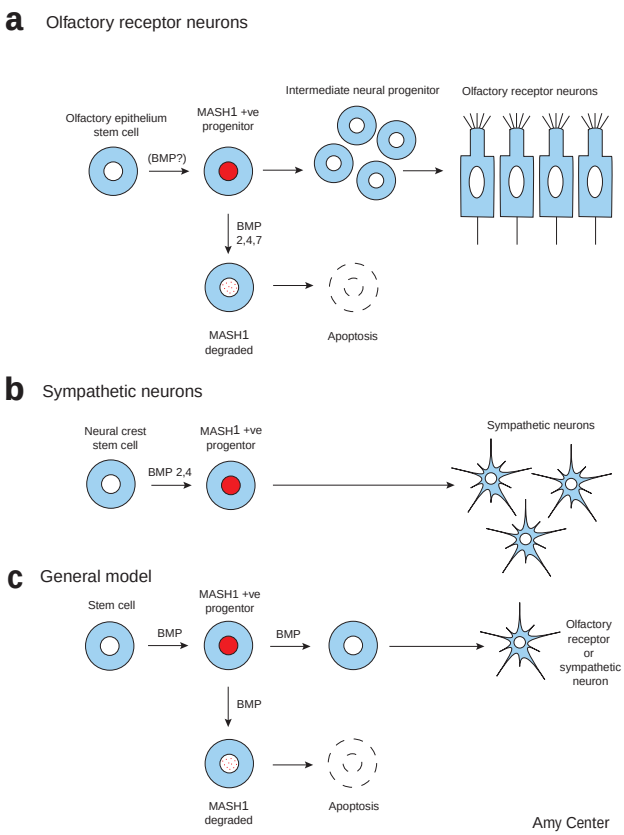

Fig. 1. Role of BMP signaling in development of olfactory and sympathetic neuronal lineages. **(a)** Olfactory epithelial stem cells give rise to a MASH1-positive progenitor, which can adopt different fates. In the presence of BMP, MASH1 is rapidly degraded, and the cell subsequently dies by apopotosis. In the absence of BMP signaling, it goes on to proliferate, forming intermediate neuronal progenitors and eventually postmitotic olfactory receptor neurons. **(b)** Neural crest stem cells are induced by some but not all BMPs to form MASH1-positive progenitors, which later downregulate MASH1 and give rise to sympathetic neurons. (In the absence of BMP signaling, the stem cells give rise to other cell types.) **(c)** The two lineages share some common features, including a requirement for MASH1 expression at an early stage, and for its downregulation at a later stage. Both lineages are regulated by BMP signaling; its different effects in the two cases may reflect subtle differences in their sensitivities to different doses at different developmental stages, rather than any fundamental difference in its mode of action.

MASH1 seems to have a similar role in both lineages, which is perhaps not surprising given the abundant evidence that similar transcription factors act to determine neural fate and identity in widely divergent phyla[10]. In both the olfactory epithelium[11] and the neural crest[12], MASH1 is expressed at an early stage and then downregulated before differention. Moreover, mice lacking MASH1 show a loss both of ORNs and of sympathetic and enteric ganglionic neurons (the latter two of which arise from the neural crest)[8]. The more surprising result is that BMP signaling seems to have opposite effects in the two lineages; in neural crest cells, it induces *MASH1* gene expression and promotes neurogenesis, whereas in olfactory epithelial cells, it induces the degradation of MASH1 protein and inhibits neurogenesis.

To make sense of this result, we must first consider the role of MASH1 in more detail. In the case of neural crest cells (which have been better studied), the downregulation of MASH1 is as critical for their terminal differentiation as its earlier expression was for their proliferation and survival. Two lines of evidence support this idea. First, when neural crest cells are infected with a retrovirus that drives MASH1 expression, they are induced to express early differentiation markers, such as Phox2a and c-RET, even in the absence of BMP signaling[13]. Yet, most of these cells do not become fully differentiated neurons; even though most of the colonies that develop from cells infected with the virus go on to develop at least some neurons, these still represent only a small proportion of the cells within a given colony. Moreover, the cells that do become neurons seem to be those that express lower levels of MASH1 (perhaps due to silencing of the viral enhancer), suggesting that the downregulation of MASH1 may be required for their differentiation[14]. A second piece of evidence comes from further analysis of the MASH1-deficient mutant mice[15]; cells

within the ventral telencephalon that would normally express MASH1 show premature differentiation, again suggesting that the continued expression of MASH1 may normally serve to inhibit progression to the fully differentiated state.

How then can we explain the apparently opposite effects of BMPs in olfactory and neural crest lineages? It is of course possible that BMPs might somehow produce opposite effects on MASH1 in each cell type. A more attractive possibility, however, is that underlying signaling pathways are fundamentally similar in the two cases (Fig. 1c), and that BMPs can induce both the appearance and the degradation of MASH1 in both lineages (David Anderson, personal communication). This would allow BMPs to act as both promoters and inhibitors of neuronal fates, depending on precisely when they act. In such a model, the choice between differentiation and death could depend on the exact timing and amount of BMP signaling relative to the progenitor cells' changing responsiveness over time.

Clearly, the function of BMPs in the olfactory epithelium is far from resolved, and the findings of Shou and colleagues raise a number of interesting questions. Does the BMP-mediated degradation of MASH1 actually cause the cessation of cell division and the onset of apoptosis? What is the molecular link between BMP signaling and the proteolysis of MASH1? Is MASH1 the only molecule targeted by BMPs for degradation, or are there others? Does the level of one or more BMP act to control the rate of olfactory neurogenesis *in vivo*? No doubt, future experiments will soon address these issues. In the interim, it seems that BMPs have once again dropped a question on our plate.

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Anandamide: a candidate neurotransmitter heads for the big leagues

David W. Self

Activation of dopamine receptors triggers release of anandamide, an endogenous cannabinoid, ***in vivo***, leading to inhibition of dopamine-mediated locomotor behavior.

Endocannabinoids are endogenous substances that mimic the psychoactive effects of marijuana on cannabinoid receptors[1]. The story of their discovery goes back to the last decade, when pharmacological and molecular studies[2,3] led to the identification of a G-protein-coupled receptor that was activated by Δ^9-tetrahydrocannabinol (Δ^9-THC), the major psychoactive substance in marijuana. Just as the existence of opioid receptors led to the discovery of endogenous opioid neurotransmitters in the 1970s[4], the identification of the brain cannabinoid receptor CB_1 spurred a search for naturally occurring ligands within the brain.

Several endogenous ligands for the CB_1 receptor have been discovered, but none has yet been shown to function as a neurotransmitter. In this issue of *Nature Neuroscience*, Giuffrida and colleagues report that local depolarization can trigger the release of anandamide, the first endocannabinoid identified, in the striatum of awake, freely moving rats[5]. They also show that anandamide release can be stimulated by dopamine receptors, and that this leads to the inhibition of dopamine-mediated locomotor behavior via cannabinoid receptors. Their findings promise to propel anandamide from candidate status to *bona fide* neurotransmitter, and may also open the door to novel treatments for diseases that involve dysfunction of dopamine signaling.

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The name anandamide is derived from the Indian Sanskrit term *ananda*, meaning 'bliss and tranquillity'[1], undoubtedly in reference to psychoactive effects of cannabinoids in humans. Anandamide belongs to a class of molecules called eicosaniods, and it was first isolated based on its hydrophobic properties, by analogy with exogenous cannabinoids such as Δ^9-THC[6]. It is expressed throughout the brain, and it is most prevalent in the hippocampus, striatum, cerebellum and cortex, structures that regulate learning, movement and cognition, among other behaviors. Another endocannabinoid, 2-arachidonylglycerol (2-AG), which was discovered more recently, is even more highly expressed in the brain[1]. Both molecules fulfill at least some of the criteria for neurotransmitter status. They both activate the brain cannabinoid receptor CB_1, and both have putative biosynthetic pathways. (They are synthesized from arachidonic acid and phospolipids.) Anandamide also has a putative mechanism for its inactivation via re-uptake and intracellular degradation. Being hydrophobic molecules, neither anandamide nor 2-AG is packaged into synaptic vesicles (in contrast to conventional neurotransmitters); instead, they are thought to be released by phospholipase-mediated cleavage followed by passive diffusion across the plasma membrane[1].

Because of their low (micromolar) affinities for the CB_1 receptor, however, many investigators were skeptical as to whether anandamide or 2-AG ever attain sufficient concentrations to activate the